PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing *(day/month/year)*	**16 FEBRUARY 2006 (16.02.2006)**
Applicant's or agent's file reference PCTA9511-715	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/KR2005/003923	**18 NOVEMBER 2005 (18.11.2005)**	23 NOVEMBER 2004 (23.11.2004)

International Patent Classification (IPC) or both national classification and IPC

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Applicant

Korea Research institute of Bioscience and Biotechnology et al

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.
For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/KR	Date of completion of this opinion	Authorized officer
Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	15 FEBRUARY 2006 (15.02.2006)	SHIN, Weon Hye Telephone No.82-42-481-5591

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/KR2005/003923

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material
   - ☒ a sequence listing
   - ☐ table(s) related to the sequence listing

   b. format of material
   - ☒ on paper
   - ☒ in electronic form

   c. time of filing/furnishing
   - ☒ contained in the international application as filed.
   - ☒ filed together with the international application in electronic form.
   - ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additioanl copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-13	YES
	Claims	none	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-13	NO
Industrial applicability (IA)	Claims	1-11	YES
	Claims	12,13 (?)	NO

2. Citations and explanations :

Reference is made to the following documents from the International Search Report (ISR):
D1: Sheng Wu Gong Cheng Xue Bao (Shen, W., CN) May 2004
D2: US 5741957 A
D3: US 6180761 A1
D4: US 2003/0024002 A1

1. Novelty

Objective of the present invention is to provide a bovine beta-casein gene targeting vector for ectopic expression of the gene for a desired protein, a bovine somatic cell generated with said vector by homologous recombination, a nuclear-transferred embryo with said somatic cell, a method for producing said bovine somatic cell and a method for generating a transgenic cattle. The present invention also provides a method for producing a desired recombinant protein from animal milk.

The subject matter of the present invention (claim 1) comprises a 5-12 kb long 5' flanking sequence including the promoter and some of the bovine beta-casein gene; a region for cloning a nucleic acid coding for a desired protein; a region for positive selection marker; and a 2.8-3.5 kb long 3' flanking sequence of the beta-casein gene.

D1(the whole text written in Chinese needs to be translated, but the abstract in English discloses details of the constituents) is considered the most relevant state of the art of the present invention in providing a gene targeting method for producing mammary gland bioreactor. To this end, D1 uses the beta-casein locus for knock-in with a gene of interest. The gene targeting vector disclosed in D1 comprises the left arm (6.3 kb long 5' flanking sequence of goat beta-casein gene); gene of interest; positive selection marker(neo); the right arm (2.4 kb long 3' flanking sequence of the beta-casein gene); the negative selection marker just outside the right arm. D1 discloses transfected cells and a method therefor. However, D1 uses the goat beta-casein locus that makes D1 different from the present invention. The abstract of D1 describes neither a nuclear-transferred embryo nor a method for generating a transgenic cattle, which produces the desired protein in milk (claims 8-13).

- continued in Supplemental box

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of :

Box V

D2 describes a means for producing a transgenic bovine using the gene targeting method by homologous recombination. However, the gene of the mammary gland disclosed in D2 is the bovine alpha S1-casein gene, even if the bovine beta-casein locus is named as an equivalent in col.14, ll. 21~34. D2, thus, does not disclose the constituents for the vector construct specific to the beta-casein gene. D2 is also different in that the methods for introducing overlapping transgene fragments into embryonal target cells include microinjection but not Somatic Cell Nuclear Transfer(SCNT).
There is no direct indication of the subject matter of claim 1 in any other prior art, either.
Accordingly, claim 1 and claims 2~13, which are referred to claim 1, appear to be novel meeting the requirements set forth in Article 33(2) PCT.

2. Inventive step
The characterizing feature of claims 1,8,9,10,12,13 over D1 is targeting the bovine beta-casein locus by SCNT. Choosing the bovine beta-casein gene, however, is unremarkable for a person skilled in the art since D2 teaches the bovine beta-casein locus as merely one of a plurality of options that the person skilled in the art would select among various mammary gland specific genes(cf. the PCT Guidelines, C-IV, 8.8(CI)(i)).
The sequence of the bovine beta-casein gene is disclosed in D3(col.1, ll.60~67). D4 relates to a method for SCNT: the genetic modifications are engineered in somatic cells cultured in vitro using the technique of gene targeting, and the genetically modified cells are then used as nuclear donors to produce transgenic animals. SCNT and gene targeting vectors for homologous recombination is thus considered customary practices in the technical field.
Therefore, it is obvious that the skilled person could arrive at the claimed invention from the teachings of D1~D4 with a reasonable expectation for success, unnecessarily carrying out undue experimentation. The advantage thus achieved is also foreseen. Therefore, claims 1,8,9,10,12,13 do not appear to involve an inventive step.
The subject matter of claims 2-7 & 11 merely add features that come within customary practices in the art.
Consequently, claims 1~13 are not considered to comply with the requirement of inventive step set out in Article 33(3) PCT.

3. Industrial applicability
(i) regarding claims 1-11:
Objective of claims 1-9 is to provide means for producing a desired proteins from milk of the transgenic animals. Claims 10 & 11 relate to methods for producing transgenic embryo by SCNT, which comprises steps to be performed in vitro. Therefore, the subject matter of claims 1-11 meets the requirements of Article 33(4) PCT.

(ii) regarding claims 12 & 13:
Claim 12 relates to a method for generating a transgenic cattle including step (5) for implanting the embryo into a recipient(cattle). Step (5) should be regarded as a method for treatment of the human and animal body by surgery [Rule 67.1(iv) PCT]. Claim 13 is referred to claim 12.
For the assessment of claims 12 & 13 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent on the formulation of the claim.